Gudding R, Lillehaug A, Midtlyng PJ, Brown F (eds): Fish Vaccinology.
Dev Biol Stand. Basel, Karger, 1997, vol 90, pp 93-105

# Immunization with Bacterial Antigens: *Vibrio* Infections

*A.E. Toranzo, Y. Santos, J.L. Barja*

Departamento de Microbiología y Parasitología, Facultad de Biología,
Universidad de Santiago de Compostela, Spain

**Key words:** Immunization; bacterial antigens; *Vibrio* infections.

**Abstract:** Within the genus *Vibrio*, the species causing the most economically important diseases in marine culture are *Vibrio anguillarum*, *V. ordalii*, *V. salmonicida* and *V. vulnificus* biotype 2. For these bacterial fish pathogens host range, clinical importance, virulence mechanisms, the antigenic variants relevant to vaccination, the existence of genetic intraspecific diversity and the available vaccines including commercial or domestically produced will be described.
Among the 10 serotypes described in *V. anguillarum*, only serotypes O1, O2 and O3 have been associated with mortality in a great variety of farmed and feral fish worldwide. Whereas serotype O1 is a very homogeneous group from the biochemical, serological and genetic stand-point, within serotype O2 and O3 two antigenic entities have been detected. Moreover these two serotypes present a remarkable genetic heterogeneity. However, many of the available commercial vibriosis vaccines include in their formulations only *V. anguillarum* serotype O1 in combination with *V. ordalii* (formerly *V. anguillarum* biotype 2). In addition no commercial vaccine provides information about the subgroup(s) used as representive of *V. anguillarum* O2.
Recently, *Vibrio* species taxonomically related to *V. anguillarum* (VAR) have been isolated from diseased fishes. An extensive characterization of these VAR organisms allowed us to distribute them into at least seven O-serogroups. The inclusion of representative VAR strains in the vibriosis vaccines need to be discussed. *V. ordalii*, *V. salmonicida* and *V. vulnificus* are homogeneous species with respect to biochemical reactions, serology and degree of virulence, possess a narrow host range and seem to be restricted to some geographic areas.
Although iron acquisition systems can be involved in the virulence mechanisms of these pathogens, only in *V. anguillarum* has it been clearly demonstrated that the ability to scavenge iron from the host is a crucial virulence determinant. The role of exotoxins and cell surface associated properties in the *Vibrio* infections remains to be elucidated.

## *Vibrio anguillarum* and *Vibrio ordalii*

Vibriosis due to *Vibrio anguillarum* is one of the most serious bacterial diseases affecting the marine culture industry worldwide. This microorganism is responsible for severe economic losses in a great variety of fish species of economic importance including Pacific salmon, Atlantic salmon, rainbow trout, turbot, seabass, seabream, striped bass, cod, Japanese and European eel, and ayu [1]. The species *V. ordalii*,

has been established to accommodate strains previously classified as *V. anguillarum* biotype 2. In contrast to *V. anguillarum*, the disease caused by *V. ordalii* has never been documented in Europe, being described only in North America, Japan and Australia.

Fish affected by the classical vibriosis show typical signs of a generalized septicaemia with haemorrhages in the base of fins, exophthalmia, and corneal opacity. Fish are frequently anorexic with pale gills which reflects a severe anaemia, and the skin becomes dark in colour. Oedematous lesions, predominantly centered on the hypodermis and extending deep into the muscle and to the epidermis are often observed. At necropsy, the liver is pale and pethechial haemorrhages are commonly found in the visceral organs and the body muscle. In some cases the abdomen is filled with fluid causing distension. In chronic cases, the severe haemolytic anaemia resulting from the effect of the lytic extracellular products causes a heavy deposition of haemosiderin in the melanomacrophages of the ellipsoids [2, 3]. In the case of disease caused by *V. ordalii*, the bacteraemia develop much later than in infections with *V. anguillarum* which might explain the lower number of bacterial cells in the blood. Another difference from classical vibriosis is the marked decrease in the number of leucocytes in the blood.

Although a total of ten O serotypes (O1-O10, European serotype designation) are known to occur among *V. anguillarum* isolates, only serotype O1 and O2 and, to a lesser extent, serotype O3, have been associated with mortality in farmed and feral fish through the world [1, 4, 5]. In contrast to serotype O1, it has been demonstrated that bacteria belonging to serotypes O2 and O3 display antigenic heterogeneity. Within serotypes O2 and O3, two different patterns of serological reactions, based on O-antigens, have been detected. These antigenic entities were designated as subgroups O2a and O2b or subgroups O2α and O2ß [1] and subgroups O3A and O3B [6, 7]. Whereas subgroup O2α occurs in both salmonid and non salmonid fish, subgroup O2ß has only been detected in marine fishes. Within the serotype O3, the subgroup O3A is recovered from diseased fish and subgroup O3B comprises only environmental strains (Table 1). Electrophoretic analysis and immunoblot assays of cell envelope components have demonstrated that strains belonging to serotype O1 possess immunologically related lipo-polysaccharide (LPS) and proteins, while *V. anguillarum* isolates grouped in serotypes O2 and O3 showed internal heterogeneity in their LPS and protein banding patterns [7] (Fig. 1a & b). Some of the remaining serotypes (O4 and O10), considered to include only environmental strains, have recently been described to be virulent for fish (serotype O4) [8] or associated with mortality of artificially reared cod (O4, O6 and O8) [5].

Although *V. anguillarum* is the best known and most widespread pathogenic *Vibrio* species, its mechanism of virulence is not fully understood. At present there are contradictory reports about the existence of host specificity among the isolates, as well as which of the classically considered virulent serotypes (O1 or O2) has the highest pathogenic potential for fish. Our infection experiments indicate that strains of both serotypes possess a similar degree of virulence for turbot, rainbow trout, Atlantic salmon and coho salmon regardless of their origin of isolation [9].

It is known that some cell-surface related components can enable bacteria to locate, adhere to, and penetrate mucosal surfaces, contributing to their rapid spread through host tissues. However, the inability to demonstrate in *V. anguillarum* any relation among hydrophobicity, adhesiveness, and strain virulence [10, 11], indicate the unreliability of these properties as virulence markers in sero-epizootiological studies.



*Table 1:* Predominant serotypes and antigenic subgroups associated with vibriosis caused by *V. anguillarum*.

| Fish species | Serotypes |
|---|---|
| Salmonids | O1 / O2α |
| Turbot | O1 / O2α / O2ß |
| Cod | O2α / O2ß |
| Striped bass | O1 / O2α / O2ß |
| Seabass | O1 / O2α / O2ß / (O3A)* |
| Seabream | O1 / O2α / O2ß |
| Ayu | O1 / O2 / O3A |
| Eel | O2 / O3A |

* Only in France.

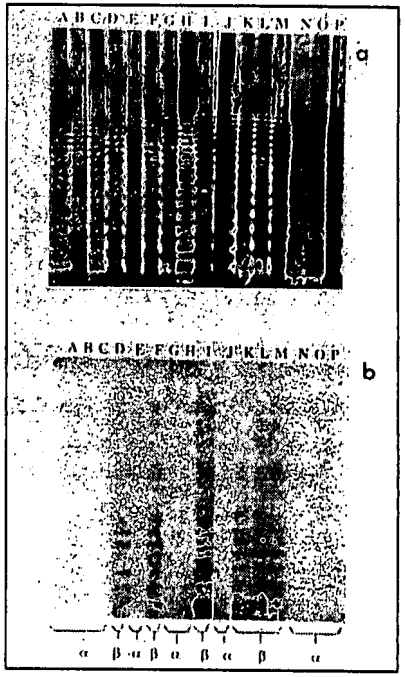


*Fig. 1:* (a) SDS-PAGE of LPS components of strains of *V. anguillarum* of serotype O2 (subgroups α and ß), and (b) the corresponding immunoblot with unabsorbed antiserum against a strain of serogroup O2ß. Lanes A, B, C, E, G, H, J, N, O, P relate to strains belonging to serotype O2α. Lanes D, F, I, K, L, M, strains belong to serotype O2ß.

The widespread tissue damage and haematological changes which accompany vibriosis have been variously attributed to endotoxin or exotoxin or both. While the role of bacterial endotoxins in the pathogenesis of vibriosis is unclear, a great number of studies have demonstrated that *V. anguillarum* extracellular products (ECP) are highly toxic for fish and possess a variety of activities including proteases, haemolysins, exohaemagglutinins, cytotoxins and dermatotoxins [12]. Our results indicated that regardless of the isolation source, serotype and degree of virulence of the strains, all the ECP possess a similar enzymatic profile and are toxic for turbot, salmon and rainbow trout with a mean lethal dose ($LD_{50}$) ranging from 4,5 to 7,3 µg ECP protein/g fish [9]. Moreover, analysis by electrophoresis and immunoblot of the ECP components has demonstrated that strains of serotypes O1, O2 and O3 produce extracellular LPS and immunologically unrelated proteins [7] (Fig. 2 a & b). In addition, a strong immunogenic relationship between the LPS present in the ECP and those from the cell envelope was observed which indicates

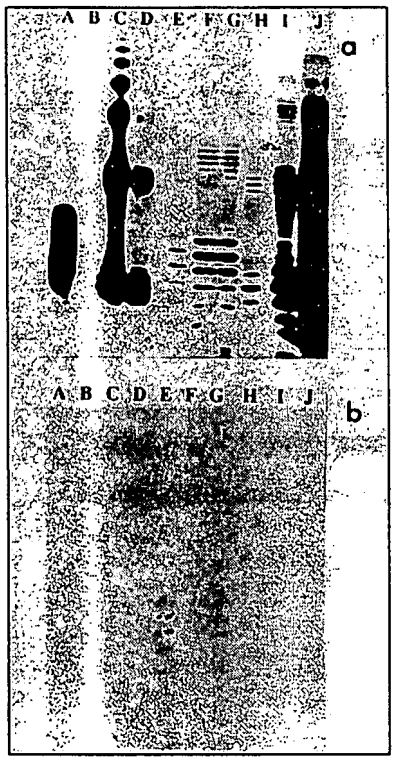


*Fig. 2:* (a) SDS-PAGE of LPS present in the ECP from *V. anguillarum* of serotypes O1, O2, and O3, and (b) the corresponding immunoblot with antiserum against the ECP from a strain of serotype O2. Lanes A,B,C.D. strains of serotype O1. Lanes E,F,G, strains of serotype O2. Lanes H,I,J, strains of serotype O3.



that the enrichment of bacterins with inactivated ECP must improve the efficacy of the *Vibrio* vaccines. This was clearly supported in experimental vaccination trials conducted in trout and turbot [13].

Iron acquisition systems play a role in the virulence mechanisms of different fish pathogens. However, *V. anguillarum* is the only fish pathogen in which it has been clearly demonstrated that the ability to scavenge iron from the host transferrins is a crucial virulence determinant. The best known mechanism for iron uptake in *V. anguillarum* involves the production of a diffusible iron chelating compound, a siderophore, and the synthesis of iron-regulated outer membrane proteins acting as receptor for the ferri-siderophores. Two different siderophore-mediated systems seem to occur. One is mediated by a 47 Md (65 Kb) plasmid (pJM1) present in most strains belonging to serotype O1, whereas the second system is chromosome-mediated and is present in all serotype O2 strains. [14-16].

The plasmid-mediated system involves a catechol-type siderophore named anguibactin and two outer membrane proteins which are synthetised under iron starvation conditions. One of these proteins, known as OM2 of 86 Kd, is plasmid-encoded and was identified as the receptor for the ferric-anguibactin. Transposition mutagenesis analysis allowed us to obtain mutants defective in siderophore synthesis, or lacking the production of the outer membrane protein OM2. These mutant strains proved to be avirulent by experimental infection in fish, which demonstrates that both components, siderophore and receptor, are essential for an efficient iron transport into the cell [17]. Another approach to obtain stable attenuated strains in *V. anguillarum* O1 was the construction of a chromosomal aromatic-dependent aro-C mutant by allelic exchange which was impaired in the synthesis of 2,3-dihydroxybenzoic acid (DHBA) that is a precursor in the biosynthesis of the plasmid-mediated siderophore anguibactin [18].

All serotype O2 strains of *V. anguillarum* also possess a siderophore-mediated iron acquisition system, but the components are different from those encoded by the virulence plasmid pJM1 [15, 16]. These strains produce a catechol-type siderophore with an unknown structure, and two main iron-regulated outer membrane proteins of 75 and 70 Kd. The protein of 75 Kd acts as the receptor of the siderophore. No genetic homology has been found between the plasmid-coded components of the anguibactin-mediated system and the chromosome-coded iron uptake system [15]. Chemical mutagenesis experiments conducted with the serotype O2 of *V. anguillarum* allowed us to obtain attenuated strains deficient in the iron uptake mechanism, which indicates that the chromosomal system is also an important virulence factor for this serotype [19].

The potential use of all these mutants for the development of a live vaccine to prevent fish vibriosis is currently under investigation. In addition, since the iron-regulated outer membrane proteins (IROMP) are strongly immunogenic, the enrichment of the *Vibrio* bacterins with these IROMPs could improve the fish protection against disease.

*Vibrio anguillarum* serotype O2 has been the most controversial component in commercial vibriosis vaccines. Many commercial vaccines are based on *V. anguillarum* O1 and *V. ordalii* as the representative for the O2 serotype (Table 2). Although cross-reactions exist between *V. ordalii* and *V. anguillarum* O2 using polyclonal antisera, studies by Svendsen & Larsen [20] using monoclonal antibodies and Mutharia et al [21] using Western immunoblot analysis with absorbed antisera demonstrated that the LPS molecules of both species do not have identical

*Table 2:* Some commercial *Vibrio* vaccines.

| Company | Vaccine | Composition |
|---|---|---|
| Rhône Merieux (France)* | Vibriffa | *V. anguillarum* O1 |
| Biovac (France) | Vibrio-vaccine | *V. anguillarum* O1 |
| AVL (United Kingdom) | Aquavac-Vibrio | *V. anguillarum* O1 / *V. ordalii* |
| | Aquavac Eurovac 5 / Vibrio | *V. anguillarum* O1 / *V. ordalii* / *A. salmonicida* |
| | Aquavac Trippel Vac | *V. anguillarum* O1 / O2 / *A. salmonicida* / *Y. ruckeri* |
| | Aquavac Multivac | *V. anguillarum* O1 / *V. ordalii* / *V. salmonicida* / *A. salmonicida* / O2 |
| Alpharma (USA) (formerly Biomed) | Biovax-1200 | *V. salmonicida* |
| | Biovax-1300 | *V. anguillarum* O1 / *V. ordalii* |
| | Biovax-1600 | *V. anguillarum* O1 / O2 / *V. salmonicida* |
| | Biojec-1800 | *V. anguillarum* O1 / O2 / *A. salmonicida* |
| | Biojec-1900 | *V. anguillarum* O1 / O2 / *V. salmonicida* / *A. salmonicida* |
| Alpharma (Norway) | Apovax HS | *V. salmonicida* |
| | Apovax duo | *V. anguillarum* O1 /O2/ *V. salmonicida* |
| | Apoject 2-fural | *V. salmonicida* / *A. salmonicida* |
| | Apojec 1800 | *V. anguillarum* O1 /O2/ *A. salmonicida* |
| | Apoject 3-fural | *V. anguillarum* O1 /O2 *V. salmonicida* / *A. salmonicida* |
| Intervet Norbio (Norway) | Norvax Vibrio 3 | *V. anguillarum* O1 /O2 / *V. salmonicida* |
| | Norvax Protec | *V. anguillarum* O1 /O2 *V. salmonicida* / *A. salmonicida* |
| | Norvax Protect IPN | *V. anguillarum* O1 /O2 *V. salmonicida* / *A. salmonicida* /IPN |
| Aqua Health Ltd (Canada) | Lipogen triple | *V. anguillarum* O1 / *V. ordalii* *V. salmonicida* / *A. salmonicida* |
| | Vibrogen | *V. anguillarum* / *V. ordalii* |
| Microtek (Canada) | Microvib | *V. anguillarum* O1 / *V. ordalii* |
| | Microvax | *V. anguillarum* O1 |
| | Microvox | *V. ordalii* |
| Kyoritsu Shoji Co. (Japan) | Piscivac VA | *V. anguillarum* O1 / *V. ordalii* |
| (Australia) | Anguillivac C | *V. anguillarum* O1 |
| University of Santiago (Spain) | GAVA-3 | *V. anguillarum* O1 / O2α / O2ß |
| University of Valencia (Spain) | Vulnivaccine | *V. vulnificus* (serotype E) |

* No longer sold.



antigenic properties. Thus, the results of these studies suggest that immunization of fish with *V. ordalii* would elicit very poor protection against infections by *V. anguillarum* O2. This was supported by the appearance of natural vibriosis outbreaks by *V. anguillarum* O2 in fish vaccinated with a bivalent commercial bacterin composed by *V. anguillarum* O1 and *V. ordalii*.

In conclusion, *V. ordalii* should not be considered relevant in vaccines used in areas such as Europe where this species has not been isolated. In addition, among the *V. anguillarum* strains, it is important to include in the vaccines representatives covering a broad antigenic spectrum, bearing in mind which fish species should be protected, what geographical zone should be covered, and which serological variety of the strains predominates in a given area. Table 3 shows the proposed vaccine formulations against the typical vibriosis, considering the fish species and the geographic area where the fish are cultured. According to these criteria: (i) in vaccines for cod and eel the inclusion of *V. anguillarum* O1 is not necessary, (ii) the serogroup O2ß is not relevant in vaccines for salmonids and (iii) for eel and ayu as well as for seabass in France, the inclusion in the vaccines of *V. anguillarum* O3A is recommended. Unfortunately, in all the commercial vaccines the subgroup employed as representative of the *V. anguillarum* O2 is not mentioned. Only the toxoid-enriched bacterin GAVA-3 produced by the University of Santiago in Spain [13] provides this information (Table 3).

Several genetic studies have been performed to study the intra-as well as inter-species relationships of the different serotypes of *V. anguillarum* and *V. ordalii*. Pedersen & Larsen [22], comparing the strains of *V. anguillarum* serotype O1 isolated from different geographical areas and from distinct fish species by rRNA gene restriction analysis (ribotyping), found a remarkable homogeneity represented by one major ribotype. Only by using pulsed-field gel electrophoresis (PFGE) could the Scandinavian strains and southern European isolates be

*Table 3:* Proposed *V. anguillarum*-*V. ordalii* vaccine formulations.

| Fish species | Geographic area | Vaccine composition |
|---|---|---|
| Salmonids | North America Japan Australia | *V. anguillarum* O1 / O2α /*V.ordalii* |
| | Europe | *V. anguillarum* O1 / O2α |
| Striped bass | USA | *V. anguillarum* O1 / O2α / O2ß |
| Turbot | Europe | *V. anguillarum* O1 / O2α / O2ß |
| Cod | North Europe | *V. anguillarum* O2α / O2ß |
| Seabass/Seabream | South Europe | *V.anguillarum* O1 /O2α / O2ß /(O3A)* |
| Eel | Europe Japan | *V. anguillarum* O2α / O2ß O3A |
| Ayu | Japan | *V. anguillarum* O1/ O2α / O2ß / O3A |

* Only for seabass reared in France.

separated [23]. In the case of *V. anguillarum* serotype O2, the strains were divided into 32 distinct ribotypes without any relationship with the subgroups O2a and O2b. In addition, a genetic difference between North European and South European O2 strains could be detected by ribotyping [24]. This difference was also observed when the plasmid profiles were studied because plasmids were practically detected only in the North European isolates. All these results support the idea of the presence of two separate clonal lineages within both serotypes of *V. anguillarum*.

In contrast, the ribotypes of *V. ordalii* were clearly different from those of *V. anguillarum* O2, with similarities between these two species being less than 40% [24]. In addition, although three ribotypes were discernible in *V. ordalii*, the genetic similarity among the strains was more than 95%. This genetic homogeneity together with the identical plasmid profiles (a 23 Md plasmid) supports the clonality of this species.

Regarding vaccination programmes, it remains to be determined whether differences in ribotypes can be associated with changes in the antigenicity of the strains.

## *Vibrio anguillarum*-Related (VAR) Organisms

Vaccines which contain preparations of *V. anguillarum* of the serotypes O1 and O2 have proved to be very effective for the control of vibriosis caused by these serotypes [13]. However, *Vibrio* species taxonomically related to *V. anguillarum* (VAR) have been isolated from vaccinated fishes [8, 25, 26] in different geographical areas. VAR strains can be differentiated from *V. anguillarum* on the basis of biochemical and serological characteristics [8, 25, 26]. Moreover, it has been demonstrated [26] that VAR strains can be typed on the basis of heat stable O antigen and that strains isolated from diseased fish can be grouped into six serogroups A, B, C, D, F and G. Serotypes C and F are predominant among strains isolated from turbot and cod, respectively. These results suggest that formulation of present vibriosis vaccines may be improved if VAR strains representatives of the antigenic groups which predominate in a particular area are tested in trial vaccines. Interestingly, whereas all the serotype F strains were virulent for fish, avirulent strains predominate in serotype C.

## *Vibrio salmonicida*

A major new disease appeared in 1979 in Norwegian salmonid farms around the island of Hitra. Since then, «Hitra disease» has been reported in salmonids and cod all along the western and northern coastlines of Norway, Scotland and Canada. The aetiology of «Hitra disease» or «coldwater vibriosis» has been disputed, with nutritional or metabolic disorders suggested as important factors predisposing of outbreaks. However, a new species *Vibrio salmonicida* has been consistently isolated from fish with coldwater vibriosis [27].

Fish infected with *V. salmonicida* are usually lethargic, dark coloured and they stop feeding. They exhibit an extensive pethechial haemorrhage in the vent region. Internally, coldwater vibriosis is characterized by severe anaemia and extensive



haemorrhages, specially in the integument surrounding the visceral organs including the caeca, abdominal fat and the kidney. The spleen is bright red and the rectal region appears haemorrhagic with watery contents. A general septicaemia with large numbers of bacteria is usually found in the blood of moribund fish. Microscopy has revealed damage of endothelial cells in the liver, spleen, nephrons, and muscle tissues [3].

All *V. salmonicida* strains are very similar with respect to biochemical reactions, serology and degree of virulence [28]. The 50% lethal doses ($LD_{50}$) obtained by intraperitoneal injection ranged from $10^6$ to $10^8$ CFU/fish, which are high values compared with those reported for *V. anguillarum*. This indicates that certain stressful environmental or nutritional conditions may lead to increased abundance or virulence of the bacterium and/or an immunological weakness of the fish.

It was demonstrated by using monoclonal antibodies that a hydrophobic antigen called VS-P1 is a dominant surface layer product of *V. salmonicida* [29] which is released during bacterial growth in diseased fish. The VS-P1 antigen is a complex of both protein and LPS molecules of about 40,000 daltons. Whether VS-P1 is related to the pathogenesis of *V. salmonicida* is not known, but it may be similar to the A-layer protein of *A. salmonicida*, which plays a role in the protection against bactericidal action of host serum.

Regarding the possible role of extracellular products of *V. salmonicida* in the development of the disease, the anaemia and haemorrhages typical of coldwater vibriosis indicate that exotoxins and cytolytic enzymes may be involved in pathogenesis of coldwater vibriosis. However, the results obtained in vitro failed to demonstrate that *V. salmonicida* produces extracellular proteolytic enzymes, cytotoxins or haemolysins [29]. Therefore, as with *V. ordalii,* which is also a poor producer of proteases and haemolysins, the cause of coldwater vibriosis symptoms is not clear.

Epidemiological studies of coldwater vibriosis in salmon and cod have focussed on the plasmid content of *V. salmonicida* [28, 30]. Although a total of 11 plasmid profiles have been observed in *V. salmonicida*, practically all of them contain a 21-24 Md plasmid. However, curing experiments [31] demonstrated that this plasmid is not required for expression of virulence.

Interestingly, a 61 Md plasmid was present exclusively in *V. salmonicida* strains originating from northern Norway [30]. With the aim of determining whether this plasmid could confer some change in antigenic properties, *Vibrio salmonicida* isolates from cod containing this 61 Md plasmid were examined serologically by means of monoclonal antibodies prepared against different epitopes on the bacteria. The results indicated the existence of a particular subtype of *V. salmonicida* in these cod isolates. However vaccination experiments in Atlantic salmon demonstrated that there are no major antigenic differences between different strains of *V. salmonicida* that have any impact on protective immunity [32]. Therefore, in the formulation of vaccines against coldwater vibriosis, the inclusion of only a single strain is needed.

In addition, vaccination of Atlantic salmon against coldwater vibriosis does not protect against outbreaks of vibriosis due to *V. anguillarum,* which indicates that the protective immunity is specific for infections with *V. salmonicida* [33]. This finding is supported by the different LPS and membrane protein patterns of the two bacteria as well as by the antibody specificities of the Atlantic salmon antiserum against the surface antigens of these two pathogens [34]. Therefore, in areas where the two *Vibrio* diseases coexist, the use of a polyvalent bacterin containing *V. salmonicida* and

the predominant serotypes and subgroups of *V. anguillarum* is encouraged. This has been taken into consideration in some commercial vibriosis vaccines (Table 2).

## *Vibrio vulnificus*

*Vibrio vulnificus* is an estuarine bacterium that comprises two biotypes. Biotype 1 is an opportunistic human pathogen producing serious wound infections and septicaemia which mainly affect immunocompromised hosts or those with hepatic disorders. The disease caused by this biotype has generally been associated with handling or ingestion of raw shellfish [35]. Biotype 2 of this species is a primary eel pathogen which seems to be restricted to the geographic areas of Japan, Taiwan and Spain [36, 37]. However, this biotype also may act as an opportunistic pathogen capable of causing infection in humans [38], thereby representing a potential health hazard for fish farmers.

Eels affected by the biotype 2 of *V. vulnificus* rest inactive at the bottom of the tanks. The external lesions appear firstly as petechiae on the abdomen, haemorrhage of the anal fin and a reddening in the opercular region. Protrusion of the rectum is also sometimes observed. The anterior part of the belly is often swollen and the pathological changes of the skin sometimes progress to large ulcers with central whitish-yellow necrotic tissue. Internally, it is observed an inflammation of tissues and intestinal canal, pale and haemorrhagic liver, swollen kidney and accumulation of ascitic fluid in the abdominal cavity [37].

Biotype 2 is biochemically homogeneous, indole production being the main trait which distinguishes both biotypes [39]. Whereas biotype 1 is antigenically diverse, with at least 10 serotypes being described, biotype 2 strains constitute a homogeneous O serogroup regardless of the geographic origin. In addition, all LPS from biotype 2 are immunologically identical and different from LPS of biotype 1 (Fig. 3). However, the two biotypes share antigenically related outer membrane proteins (OMPs) [39]. On the basis of these data, it was proposed that biotype 2 of *V. vulnificus* constitutes an LPS-based O serogroup within this species, being designated serotype E (for eels) [39]. Preliminary dot blot analysis seems to indicate that two subgroups can be established among the biotype 2 strains, namely European isolates and Japanese isolates. However, more assays using absorbed antisera are necessary to confirm this assumption.

Both biotypes of *V. vulnificus* share several virulence determinants, with capsule, iron and exotoxins playing similar roles in pathogenicity [40]. Whereas a lack of extrachromosomal elements was described in biotype 1, all biotype 2 isolates contain at least two high MW plasmids, the function of which is still unknown. Moreover, the plasmid profiles of European eel isolates were different from those of the Japanese strains [39].

No commercial vaccines have been manufactured to prevent this disease. Only the University of Valencia (Spain) has recently produced a toxoid enriched bacterin (Table 2) containing capsulated Spanish and Japanese strains of *V. vulnificus* which proved to be very effective in both experimental and field conditions.

## Conclusions

Although promising results seem to be achieved with many commercial vibriosis vaccines, their efficacy could be improved if, in the selection of strains for vaccine formulation, the serotypes and antigenic subgroups predominating in the




*Fig. 3:* Immunoblot of isolated LPS of strains of *V. vulnificus* biotypes 1 and 2 using antiserum against a strain of biotype 2 . Lanes A, B, C, D, I, strains of biotype 2. Lanes E, F, G, H, strains of biotype 1.

fish species cultured in a particular area are carefully considered. More epide-miological studies should be made to determine the possible changes in the serotypes/subgroups of the *Vibrio* strains over the years. In addition, it is necessary to continue the genetic studies as well as the analysis of virulence determinants of the strains to improve the existing bacterins and to develop stable attenuated strains to be used as live vaccines in the field.

## Acknowledgements

The authors thank the different individuals and companies who kindly provided us with some of the information included in this review.

## References


1 Toranzo AE, Barja JL: A review of the taxonomy and seroepizootiology of *Vibrio anguillarum*, with special reference to aquaculture in the northwest of Spain. Dis Aquat Org 1990;9:73-82.

2 Devesa S, Toranzo AE, Barja JL: First report of vibriosis in turbot (*Scophthalmus maximus*) cultured in Northwestern Spain, in Ellis AE (ed): Fish and Shellfish Pathology. London, Academic Press, 1985, pp 131-140.

3 Bruno D, Poppe TT: A colour atlas of salmonid diseases. London, Academic Press,1996.

4 Tajima K, Ezura Y, Kimura T: Studies on the taxonomy and serology of causative organisms of fish vibriosis. Fish Pathol 1985;20:131-142.

5 Larsen JL, Pedersen K, Dalsgaard I: *Vibrio anguillarum* serovars associated with vibriosis in fish. J Fish Dis 1994;17:259-267.

6 Olsen JE, Larsen JL: Ribotypes and plasmid contents of *Vibrio anguillarum* strains in relation to serovar. Appl Environ Microbiol 1993;59:3863-3870.

7 Santos Y, Pazos F, Bandín I, Toranzo AE: Analysis of antigens present in the extracellular products and cell surface of *Vibrio anguillarum* serotypes O1, O2 and O3. Appl Environ Microbiol 1995;61:2493-2498.

8 Pazos F, Santos Y, Magariños B, Bandín I, Núñez S, Toranzo AE: Phenotypic characteristics and virulence of *Vibrio anguillarum*-related organisms. Appl Environ Microbiol 1993;59:2969-2976.

9 Santos Y, Lallier R, Bandín I, Lamas J, Toranzo AE: Susceptibility of turbot (*Scophthalmus maximus*), salmon (*Oncorhynchus kisutch*), and rainbow trout (*O. mykiss*) to *Vibrio anguillarum* live cells and their exotoxins. J Appl Ichthyol 1991;7:160-167.

10 Larsen JL, Rasmussen HB, Dalsgaard I: Study of *Vibrio anguillarum* strains from different sources with emphasis on ecological and pathobiological properties. Appl Environ Microbiol 1988;54:2264-2267.

11 Santos Y, Bandín I, Nieto TP, Barja JL, Toranzo AE, Ellis AE: Cell-surface-associated properties of fish pathogenic bacteria. J Aquat Anim Health 1991;3:297-301.

12 Toranzo AE, Barja JL: Virulence factors of bacteria pathogenic for coldwater fish. Ann Rev Fish Dis 1993;3:5-36.

13 Santos Y, Bandín I, Núñez S, Gravningen K, Toranzo AE: Protection of turbot, *Scophthalmus maximus* (L.), and rainbow trout, *Oncorhynchus mykiss* (Richardson), against vibriosis using two different vaccines. J Fish Dis 1991;14:407-411.

14 Crosa JH: A plasmid associated with virulence in the marine fish pathogen *Vibrio anguillarum* specifies an iron-sequestering system. Nature Lond 1980;284:566-568.

15 Lemos ML, Salinas P, Toranzo AE, Barja JL, Crosa JH: Chromosome-mediated iron-uptake system in pathogenic strains of *Vibrio anguillarum*. J Bacteriol 1988;170:1920-1925.

16 Conchas RD, Lemos ML, Barja JL, Toranzo AE: Distribution of plasmid- and chromosome-mediated iron uptake systems in *Vibrio anguillarum* strains of different origin. Appl Environ Microbiol 1991;57:2956-2962.

17 Wolf MK, Crosa JH: Evidence for the role of a siderophore in promoting *Vibrio anguillarum* infections. J Gen Microbiol 1986;132:2949-2952.

18 Chen Q, Actis LA, Tolmasky ME, Crosa JH: Chromosome-mediated 2,3-dihydroxybenzoic acid is a precursor in the biosynthesis of the plasmid-mediated siderophore anguibactin in *Vibrio anguillarum*. J Bacteriol 1994;176:4226-4234.

19 Conchas RD, Toranzo AE, Mazoy R, Lemos ML: Loss of virulence in *Vibrio anguillarum* O2 strains defective in the chromosomal iron uptake system. Abstract 5th International Colloquium on Pathology in Marine Aquaculture (PAMAQ 5), 1992, p 37.

20 Svendsen I, Larsen JL: Monoclonal antibodies against surface antigens of *Vibrio anguillarum* serogroup 02. Acta Vet Scand 1988;29:363-368.

21 Mutharia LW, Raymond BT, Dekievit TR, Stevenson RMW: Antibody specificities of polyclonal rabbit and rainbow trout antisera against *Vibrio ordalii* and serotype O2 strains of *Vibrio anguillarum*. Can J Microbiol 1992;39:492-499.





22 Pedersen K, Larsen JL: rRNA gene restriction patterns of *Vibrio anguillarum* serogroup O1. Dis Aquat Org 1993;16:121-126.

23 Skov MN, Pedersen K, Larsen JL: Comparison of pulsed-field gel electrophoresis, ribotyping, and plasmid profiling for typing of *Vibrio anguillarum* serovar O1. Appl Environ Microbiol 1995;61:1540-1545.

24 Tiainen R, Pedersen K, Larsen JL: Ribotyping and plasmid profiling of *Vibrio anguillarum* serovar O2 and *Vibrio ordalii*. J Appl Bacteriol 1995;79:384-392.

25 Myhr E, Larsen JL, Lillehaug A, Gudding R, Heum M, Håstein T: Characterization of *Vibrio anguillarum* and closely related species isolated from farmed fish in Norway. Appl Environ Microbiol 1991;57:2750-2757.

26 Santos Y, Pazos F, Toranzo AE: Biochemical and serological analysis of *Vibrio anguillarum* related organisms. Dis Aquat Org 1996;26:67-73.

27 Egidius E, Wiik R, Andersen K, Hoff KA, Hjeltnes B: *Vibrio salmonicida* sp. nov., a new fish pathogen. Intern J System Bacteriol 1986;36:518-520.

28 Sørum H, Hvaal AB, Heum M, Daae FL, Wiik R: Plasmid profiling of *Vibrio salmonicida* for epidemiological studies of cold-water vibriosis in Atlantic salmon (*Salmo salar*) and cod (*Gadus morhua*). Appl Environ Microbiol 1990;56:1033-1037.

29 Hjelmeland K, Stensvåg K, Jørgensen T, Espelid S: Isolation and characterization of a surface layer antigen from *V. salmonicida*. J Fish Dis 1988;11:197-205.

30 Wiik R, Andersen K, Daae FL, Hoff KA: Virulence studies based on plasmid profiles of the fish pathogen *Vibrio salmonicida*. Appl Environ Microbiol 1989;55:819-825.

31 Valla S, Frydenlund K, Coucheron DH, Haugan K, Johansen B, Jørgensen T, Knudsen G, Strøm A: Development of a gene transfer system for curing of plasmids in the marine fish pathogen *Vibrio salmonicida*. Appl Environ Microbiol 1992; 58:1980-1985.

32 Lillehaug A, Sørum RH, Ramstad A: Cross-protection after immunization of Atlantic salmon, *Salmo salar* L., against different strains of *Vibrio salmonicida*. J Fish Dis 1990;13:519-523.

33 Lillehaug A: A field trial of vaccination against cold-water vibriosis in Atlantic salmon (*Salmo salar* L.). Aquaculture 1990;84:1-12.

34 Bøgwald J, Stensvåg K, Hoffman J, Jørgensen T: Antibody specificities in Atlantic salmon, *Salmo salar* L., against the fish pathogens *Vibrio salmonicida* and *Vibrio anguillarum*. J Fish Dis 1991;14:79-87.

35 Tacket CO, Brenner F, Blake PA: Clinical features and an epidemiological study of *Vibrio vulnificus* infections. J Infect Dis 1984;149:558-561.

36 Tison DL, Nishibuchi M, Greenwood JD, Seidler RJ: *Vibrio vulnificus* biogroup 2: New biogroup pathogenic for eels. Appl Environ Microbiol 1982;44:640-646.

37 Biosca EG, Amaro C, Esteve C, Alcaide E, Garay E: First record of *Vibrio vulnificus* biotype 2 from diseased European eel (*Anguilla anguilla* L). J Fish Dis 1991;14:103-109.

38 Veenstra J, Rietra JPGM, Stoutenbeek CP, Coster JM, De Hier HHW, Dirsks-go S: Infection by an indole-negative variant of *Vibrio vulnificus* transmitted by eel. J Infect Dis 1992;16:209-210.

39 Biosca EG, Oliver JD, Amaro C: Phenotypic characterization of *Vibrio vulnificus* biotype 2, a lipopolysaccharide-based homogeneous O serogroup within *Vibrio vulnificus*. Appl Environ Microbiol 1996;62:918-927.

40 Amaro C, Biosca EG, Fouz B, Toranzo AE, Garay E: Role of iron, capsule, and toxins in the pathogenicity of *Vibrio vulnificus* biotype 2 for mice. Infect Immun 1994;62:759-763.



Dr. A.E. Toranzo, Departamento de Microbiología y Parasitología, Facultad de Biología, Universidad de Santiago, 15706 Santiago de Compostela, Spain.
E-mail: mpaetjlb@usc.es

Fish Vaccinology

# Developments in Biological Standardization

## Vol. 90

This series Dev Biol Stand begins with Vol. 23 and is the continuation of both «progress in Immunobiological Standardization, Vols 1-5» and «symposia series in Immunobiological Standardization, Vols 1-22».



KARGER Basel · Freiburg · Paris · London · New York · New Delhi · Bangkok · Singapore · Tokyo · Sydney

# Fish Vaccinology

## Soria Moria Hotel, Oslo, Norway
## June 5-7, 1996

Volume Editors

*Roar Gudding*
National Veterinary Institute, Oslo, Norway

*Atle Lillehaug*
National Veterinary Institute, Oslo, Norway

*Paul Midtlyng*
National Centre for Veterinary Contract Research and Commercial Services, Ltd. (VESO AS), Oslo, Norway

*Fred Brown*
Plum Island Disease Center, Greenport NY, USA

Proceedings of a Symposium organized and sponsored by the International Association of Biological Standardization (IABS), National Centre for Veterinary Contract Research and Commercial Services, Ltd. (VESO AS) and National Veterinary Institute

31 figures and 47 tables, 1997

KARGER Basel · Freiburg · Paris · London · New York · New Delhi · Bangkok · Singapore · Tokyo · Sydney